American Journal of Medical Genetics 63:318–322 (1996)

# Detection of a Novel Mutation in the SRC Homology Domain 2 (SH2) of Bruton's Tyrosine Kinase and Direct Female Carrier Evaluation in a Family With X-Linked Agammaglobulinemia

**Volker Schuster, Silvia Seidenspinner, and Hans Wolfgang Kreth**
*Children's Hospital, University of Würzburg, D-97080 Würzburg, Germany*

**X-linked agammaglobulinemia (XLA) is an inherited immunodeficiency disease with a block in differentiation from pre-B to B cells resulting in a selective defect in the humoral immune response. Affected males have very low concentrations of serum immunoglobulins leading predominantly to recurrent bacterial infections beginning at age 6 to 18 months. The gene responsible for XLA was identified recently to encode a cytoplasmatic tyrosine kinase (Bruton's tyrosine kinase, BTK). We have analyzed the BTK gene in a large family in which two brothers presented with the severe phenotype of XLA. Genomic DNA of affected boys and from healthy relatives was amplified by PCR with primers specific for the putative promoter region and for all 19 exons, including flanking intron boundaries, and subsequently screened for mutations using single strand conformation polymorphism (SSCP) analysis. Altered single strand band patterns were found using primers specific for exon 10, 15, and 18. Direct cycle-sequencing of these BTK segments detected two known polymorphisms in intron 14 and in exon 18. Sequencing of exon 10 from two boys with XLA demonstrated a novel point mutation in the SH2 domain of BTK. Direct identification of healthy female carriers in three generations was performed by amplification mutagenesis using PCR with a modified first primer. This method can easily be applied also to prenatal diagnosis.**


Received for publication January 3, 1996.
Address reprint requests to Dr. Volker Schuster, Children's Hospital, University of Würzburg, Josef-Schneider-Straße 2, 97080 Würzburg, Germany.

Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.



**KEY WORDS: Bruton's tyrosine kinase (BTK), X-linked agammaglobulinemia (XLA), carrier analysis, single strand conformation polymorphism (SSCP), amplification mutagenesis**

## INTRODUCTION

X-linked agammaglobulinemia (XLA; MIM 30030) is a rare inherited immunodeficiency disease with an estimated birth prevalence of 1–5 x $10^{-6}$ characterized by a block in differentiation from pre-B to B cells resulting in a selective defect in the humoral immune response [Bruton 1952]. Affected males have absent or very low numbers of B cells and extremely low concentrations of serum immunoglobulins. This leads to recurrent bacterial infections starting typically in early childhood [Lederman et al., 1985]. Female carriers are fully immunocompetent due to a skewed inactivation of the defective X chromosome in their $CD19^+$ B cells [Conley et al., 1985; Fearon et al., 1987; Conley and Puck, 1988; Allen et al., 1994]. The XLA gene locus has been mapped to Xq21.3-Xq22 by linkage analysis [Kwan et al., 1986; Mensink et al., 1986; Malcolm et al., 1987; Guioli et al., 1989; Kwan et al., 1990]. No recombination was observed, in any family, between the polymorphic marker DXS178 and XLA [Guioli et al., 1989; Kwan et al., 1990]. The gene responsible for XLA was identified recently to encode a cytoplasmatic tyrosine kinase (Bruton's tyrosine kinase, BTK) [Vetrie et al., 1993; Tsukada et al., 1993]. The XLA gene encompasses 37 kb and contains 19 exons [Rohrer et al., 1994]. The BTK protein can be subdivided into five functional domains: a pleckstrin-homology (PH) domain, a tec-homology (TH) domain, a Src homology 3 (SH3) domain, a Src homology 2 (SH2) domain, and a tyrosine kinase catalytic domain [Vihinen et al., 1995]. An international XLA-mutation database (BTKbase) was established in 1994 in order to facilitate analysis of BTK mutations: So far, more than 122 unique molecular events have been described in patients with XLA

[Vihinen et al., 1995]. Single base-pair substitutions comprise the most common mutational event, followed by non-sense and splice-site mutations. Furthermore, the frequency of XLA-causing mutations was shown to be approximately proportional to the length of each BTK domain, except for the TH domain [Vihinen et al., 1995].

Here we describe a novel mutation in exon 10 of the BTK gene in two brothers with the severe XLA phenotype. By direct mutation analysis using PCR amplification mutagenesis we were able to identify five additional healthy female carriers in the family.

## MATERIALS AND METHODS

### Patients

The first propositus (Fig.1; subject IV-1) is a currently 17.5-year-old man with a history of recurrent bronchitis and otitis media since age 6 months. At 5 years, when admitted to the children's hospital of Würzburg University because of *Campylobacter jejuni* enteritis, severe hypogammaglobulinemia was noted. IgG was 170 mg/dl (normal range for age: 640–1,420 mg/dl), IgA 7.0 mg/dl (52–220), and IgM 27 mg/dl (40–180). Secretory IgA in saliva was absent. The boy had received regular immunizations with DT and polio; however, no specific antibodies could be detected. The lymphocyte count was $3 \times 10^9$/L with the following distribution of lymphocyte subsets: T cells ($CD3^+$) 78%, T helper cells ($CD4^+$) 49%, cytotoxic T cells ($CD8^+$) 27%, and B cells ($CD19^+$) < 1%. Further immunological evaluation documented normal T cell functions. A tentative diagnosis of X-linked agammaglobulinemia (XLA) was made because of absence of circulating B cells and total lack of specific antibodies. Following diagnosis the boy received regular intravenous immunoglobulin infusions (about 400 mg IgG/kg body weight every 4 weeks). Despite this treatment he developed severe pulmonary fibrosis and bronchiectasis during the following years.

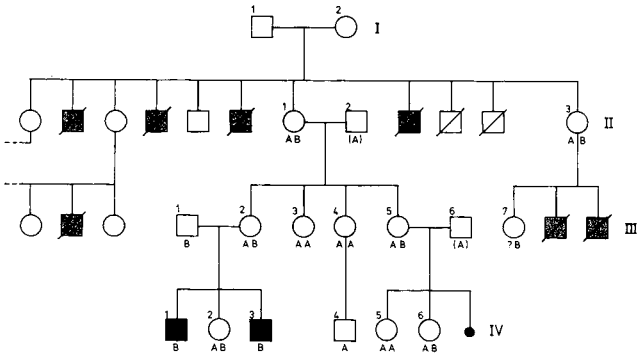



Fig. 1. Pedigree of a family with XLA. The two affected males (subjects IV-1 and IV-3) are indicated by black squares. Gray squares refer to male individuals who died during early infancy. A and B indicate the genotypes of the polymorphic marker DXS178. Subject III-2 is a probable female carrier. Since XLA could be diagnosed only in two affected brothers (IV-1, IV-3), other potential female carriers cannot be identified by using indirect linkage analysis alone. No DNA was available from the great-grandparents (I-1, I-2).

The second propositus (Fig.1, subject IV-3), a currently 11-year-old boy, also has agammaglobulinemia. Since his brother's condition was known, hypogammaglobulinemia was diagnosed at age 4 months. At this time serum IgG was 175 mg/dl (normal range for age: 190–860), IgA 8 mg/dl (10–96), and IgM 20 mg/dl (36–104). Phenotypic analysis of lymphocyte subsets revealed very low numbers of B cells (3%), while percentages of T helper cells (64%) and cytotoxic T cells (16%) were within normal limits. There was no specific antibody response to natural antigens (e.g., Candida albicans, food antigens) and after immunization with DT and inactivated polio vaccine. The boy responded well to intravenous immunoglobulin substitution and has been doing well except for repeated episodes of otitis media and diarrhea.

Several male relatives have died during infancy between the age of 6 months and 2 years, some of them because of severe bacterial infections (Fig.1, gray symbols). Laboratory data, especially serum immunoglobulin levels or numbers of peripheral B cells, are not available on these individuals.

### Linkage Analysis

Genomic DNA from 15 relatives was prepared from whole blood, digested with *TaqI* (or *PstI*, for DXS94), electrophoresed in 0.8% agarose gels and vacuum-blotted onto a nylon membrane (Gene Screen Plus membranes, Du Pont, Braunschweig, FRG). Subsequently, the membranes were hybridized with the $^{32}$P-labeled polymorphic X-chromosomal DNA markers DXS3, DXS178, DXS94, and DXS17 which are all in the vicinity of the XLA gene locus (Xq21.3-Xq22) [Kwan et al., 1986; Mensink et al., 1986; Malcolm et al., 1987; Guioli et al., 1989; Kwan et al., 1990].

### Mutation Analysis

To identify the BTK mutation in this XLA family genomic DNA from affected males (Fig.1, subjects IV-1 and IV-3) and from healthy relatives was amplified by PCR using a set of primer pairs flanking all 19 exons including intron boundaries and the putative promoter region as described previously [Vorechovsky et al., 1995]. For single-strand conformation polymorphism (SSCP) analysis the amplified PCR products were electrophoresed in a nondenaturing 5% polyacrylamide gel (Roth, Karlsruhe, FRG) containing 5% glycerol in 0.5XTBE buffer at room temperature for 7 h at 10 W. The gel was dried and exposed to Kodak XAR-5 films. Amplified PCR products from XLA patients which showed an altered single strand conformation pattern when compared to the corresponding DNA fragment of a healthy control were purified with MicroSpin™ Columns (Pharmacia, Freiburg, FRG) and directly cycle-sequenced by the dideoxy-termination method using $^{35}$S-dATP and the Exo(-)Pfu Cyclist™ DNA Sequencing Kit (Stratagene, Heidelberg, FRG) according to the manufacturer's protocol. Samples were electrophoresed on 6% polyacrylamide/8.3 M urea sequencing gels (Roth, Karlsruhe), dried and exposed to Kodak XAR-5 films.

### Direct Analysis of XLA Female Carriers by Amplification Mutagenesis

Genomic DNA from all accessible relatives and from healthy unrelated subjects were amplified by PCR with the following primer pair flanking the mutation in exon 10: 5′-CGGAGTCAGGCTGAGCAACGG-3′ and 5′-TGCAAGGAGAATGCTGTGTGC-3′. The first primer has a single sequence mismatch at position 1013 (T→G) that results in the introduction of an artificial *HaeIII* site in the mutant exon 10 allele only. Amplified PCR products were digested with *HaeIII* and electrophoresed in a 3% agarose gel and visualized by ethidium bromide staining.

## RESULTS

Linkage analysis was performed with the XLA gene-flanking markers DXS3, DXS178, DXS94 and DXS17. DXS3, DXS94, and DXS17 were not informative in this family (data not shown). The genotypes for DXS178 are indicated as A and B (Fig.1). XLA and the genotype B were coinherited by the probable carrier III-2 and passed on to the two sons with severe XLA phenotype (propositi IV-1 and IV-3). The daughter (IV-2) is most probably not a carrier, since she received the genotype B from her healthy father (III-1) and the genotype A from her mother.

In case, that subject II-1 would be an XLA carrier, subjects III-5 and IV-6 are also carriers with a high probability since they both received the genotype B. In addition, carrier status would be unlikely for all subjects who did *not* receive the genotype B (subjects III-3, III-4, and IV-5).

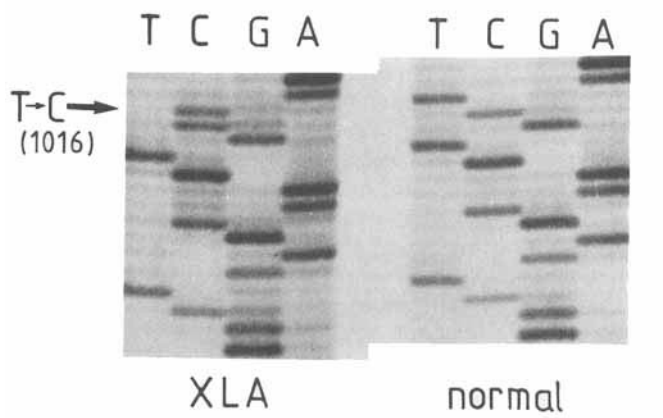
However, since there are only two brothers with diagnosed XLA in the pedigree, indirect linkage analysis alone cannot identify obligate female carriers in other parts of this family.

In order to identify the mutation responsible for the severe XLA phenotype, we analyzed the entire coding sequences of BTK including the putative promoter region and intron splize sites by single strand conformation polymorphism (SSCP) analysis. Altered single strand band patterns were found using primers specific for exon 10, 15 and 18 (data not shown). Direct cycle-sequencing of these BTK segments detected an intronic polymorphism (T→C) at position -29 of the 3' splice site of intron 14 as well as a polymorphism (C→T) at position 2031 in exon 18, which did not lead to an amino acid exchange (data not shown). Recently these polymorphisms were also identified in other families [Bradley et al., 1994; de Weers et al., 1994; Vorechovsky et al., 1995]. Sequencing of exon 10 from both XLA patients (subjects IV-1 and IV-3) demonstrated a point mutation (C*T*A→C*C*A) at position 1016, leading to an amino acid substitution at position 295 (Leucin→Prolin) (Fig. 2). In contrast, sequencing of the corresponding exon 10 segment from the healthy father (III-1) as well as from 50 unrelated healthy male subjects showed only the normal wildtype sequence.

This new mutation, designated L295P, lies in a hydrophobic cluster region of the Src homology 2 (SH2) region of BTK. It does not directly affect any of the known phosphotyrosine binding sites [Vihinen et al., 1994].

In order to determine directly the possible carrier status of females other than the mother of the two propositi, we amplified the genomic DNA of these subjects with primers flanking the point mutation in exon 10. The first primer was modified by a single nucleotide mismatch that results in the introduction of an artificial *HaeIII* site only into the mutant exon 10 sequence. Accordingly, as shown in Figure 3, females II-1 (grandmother; lane 15), II-3 (lane 16), III-2 (mother; lane 3), III-5 (aunt; lane 12), III-7 (lane 17), and IV-6 (cousin; lane 14) have both the mutant and wildtype exon 10 allele and are therefore XLA carriers. The following male and female subjects of this family [as well as 20 healthy unrelated subjects (data not shown)] had the normal wildtype exon 10 alleles: Subjects III-1 (father; lanes 1 and 2), III-3 (aunt; lane 9), III-4 (aunt; lane 10), IV-4 (cousin; lane 11), and IV-5 (cousin; lane 13). These results are in complete agreement with indirect linkage analysis (Fig.1).

The source of the BTK mutation in this family is not known. In case that those male subjects who died early in infancy (Fig. 1; gray symbols) had also been carriers of the mutant XLA gene, the mutation may have origi-




***Wildtype:***

293
Gln Leu Leu Lys Gln
1009
CAA CTG CTA AAG CAA

***Mutant:***

293
Gln Leu Pro Lys Gln
1009
CAA CTG CCA AAG CAA

Fig. 2. BTK exon 10 sequence analysis (coding strand is shown).Top, left: Part of the XLA patient's BTK exon 10 sequence with a point mutation at position 1016 (T→C) leading to an amino acid exchange at position 295 (Leucin→Prolin). Top, right: Normal (wildtype) exon 10 sequences from a healthy family member (III-1, father). Below: Wildtype and mutant exon 10 DNA sequences as well as the encoded amino acids including their position numbers.

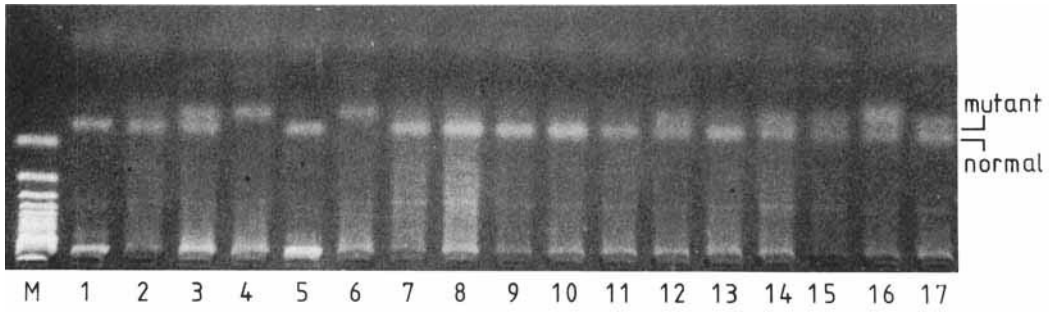



Fig. 3. Direct carrier detection in the XLA family by amplification mutagenesis. A part of BTK exon 10 containing the XLA mutation was amplified by PCR as described in material and methods. The first primer has a single base mismatch near the 3'end that results in the introduction of an artificial *HaeIII* site into the *mutant* allele only. PCR products were digested with *HaeIII* and then electrophoresed in a 3% agarose gel. The shorter (*HaeIII*-digested) mutant DNA fragment has a size of 51 bp, the larger (uncut) PCR product of the wildtype DNA fragment has a size of 72 bp. **Lane M**: 100 bp ladder; **lane 1**: subject III-1 (healthy father of two XLA patients); uncut PCR fragment; **lane 2**: subject III-1; PCR fragment after *HaeIII* digestion; **lane 3**: III-2 (mother of the two XLA patients, XLA carrier); PCR fragments after *HaeIII* digestion; **lane 4**: XLA patient IV-1; *HaeIII*-digested PCR fragment; **lane 5**: XLA patient IV-1; uncut PCR product; **lane 6**: XLA-patient IV-3; *HaeIII*-digested PCR fragment; **lane 7**: XLA patient IV-3; uncut PCR product; **lane 8**: subject IV-2 (sister); **lane 9**: subject III-3 (aunt); **lane 10**: subject III-4 (aunt); **lane 11**: subject IV-4 (cousin); **lane 12**: subject III-5 (aunt); **lane 13**: subject IV-5 (cousin); **lane 14**: subject IV-6 (cousin); **lane 15**: subject II-1 (grandmother); **lane 16**: II-3 (grandaunt); **lane 17**: III-7. Both XLA patients (IV-1, IV-3) exhibited the mutant exon 10 fragment (lane 4, lane 6). The females II-1 (lane 15), II-3 (lane 16), III-2 (lane 3), III-5 (lane 12), III-7 (lane 17) and IV-6 (lane 14) have both the mutant and wildtype exon 10 allele; they are therefore XLA carriers. Subjects III-1 (lanes 1 and 2), III-3 (lane 9), III-4 (lane 10), IV-2 (lane 8), IV-4 (lane 11), and IV-5 (lane 13) revealed the normal wildtype exon 10 alleles.

nated from one of the great-grandmaternal X chromosomes (I-2, Fig. 1) or even one generation before in the germ cell of the healthy great-great-grandfather (male mosaicism). Unfortunately, no DNA was available from these subjects.

## DISCUSSION

In this study we have identified a novel mutation (L295P) in the SH2 domain of BTK in the DNA of two brothers both presenting with the severe XLA phenotype. Analogous to related SH2 structures of other protein kinases such as Src, the SH2 domain of BTK is responsible for binding to phosphotyrosine-containing proteins [Vihinen et al., 1994].

Nineteen individual mutation types have been characterized so far in the SH2 domain [Jin et al., 1995; Vihinen et al., 1995]. Some of them, such as mutations R288W and R307G, directly affect the phosphotyrosine binding sites leading to the severe form of XLA. The L295P mutation found in this XLA family lies in a hydrophobic cluster region of SH2 [Vihinen et al., 1994]. The pathophysiologic effects might therefore be structural.

In general, no strict correlations seem to exist between the type of BTK mutation and the severity of the disease [Hagemann et al., 1995]. Clinical variability has been reported to exist even within some XLA families [Buckley et al., 1968; Wedgewood and Ochs, 1980; Leickley and Buckley, 1986]. It has been suggested that functional redundancy of other protein kinases may account for the less severe XLA phenotype found in some affected family members [Hagemann et al., 1995].

We have shown that identification of female carriers is easily possible by direct mutation analysis by using amplification mutagenesis. This applies especially in those parts of the family which are not accessible by indirect linkage analysis alone. This method needs only little amounts of genomic DNA and is therefore also well suited for prenatal diagnosis. However, this test presupposes that the BTK mutation has been already identified in one individual of the XLA family under study. Previous assays for female carrier evaluation in sporadic or unclear cases involved X chromosome inactivation studies in peripheral B lymphocytes [Conley and Puck, 1988; Allen et al., 1994]. These assays need high numbers of cells and are time consuming. However, by using this method, XLA carriers can be detected also in case that the exact BTK mutation is not known.

In the family reported here the XLA mutation appears to have originated from one of the great-grandmaternal X chromosomes (I-2), on the assumption that some of the male subjects of the next (2nd) generation, who died early in infancy also carried the XLA mutation. However, it is well possible and even more likely that the XLA mutation had already occurred one generation before in the germ cells of the healthy great-great-grandfather (X-chromosomal germ cell mosaicism). Others have demonstrated a paternal origin of the XLA mutation (male mosaicism) in five of ten XLA families examined so far, suggesting a high frequency of this phenomenon [Hendriks et al., 1989; Hagemann et al., 1995].

Further characterization of BTK mutations in XLA patients as well as in vitro studies about the complex physiological functions of the normal BTK product may not only help to better understand B cell signalling events but may also help to develop new therapeutic means (such as gene therapy) in the future.

## ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (grant Schu 560/2-3).

## REFERENCES

Allen RC, Nachtman RG, Rosenblatt HM, Belmont JW (1994): Application of carrier testing to genetic counseling for X-linked agammaglobulinemia. Am J Hum Genet 54:25-35.

Bradley LA, Sweatman AK, Lovering RC, Jones AM, Morgan G, Levinsky RJ, Kinnon C (1994): Mutation detection in the X-linked agammaglobulinemia gene, BTK, using single strand conformation polymorphism analysis. Hum Mol Genet 3:79–83.

Bruton OC (1952) Agammaglobulinemia. Pediatrics 9:722–728.

Buckley R, Sidbury JB Jr (1968): Hereditary alterations in the immune response: coexistence of "agammaglobulinemia," acquired hypogammaglobulinemia and selective immunoglobulin deficiency in a sibship. Pediatr Res 2:72–84.

Conley ME, Brown P, Packard AR, Buckley RH, Miller DS, Raskind WH, Singer JW, Fialkow P (1985): Expression of the gene defect in X-linked agammaglobulinemia. N Engl J Med 315:564–567.

Conley ME, Puck JM (1988): Carrier detection in typical and atypical X-linked agammaglobulinemia. J Pediatr 112:688–694.

de Weers M, Mensink RG, Kraakman ME, Schuurman RK, Hendriks RW (1994): Mutation analysis of the Bruton's tyrosine kinase gene in X-linked agammaglobulinemia: Identification of a mutation which affects the same codon as is altered in immunodeficient *xid* mice. Hum Mol Genet 3:161–166.

Fearon ER, Winkelstein JA, Civin CI, Pardoll DM, Vogelstein B (1987): Carrier detection in X-linked agammaglobulinemia by analysis of X chromosome inactivation. N Engl J Med 316: 427–431.

Guioli S, Arveiler B, Bardoni B, Notarangelo LD, Panina P, Duse M, Ugazio A, Oberlé I, de Saint Basile G, Mandel JL, Camerino G (1989): Close linkage of probe p212 (DXS178) to X-linked agammaglobulinemia. Hum Genet 84:19–21.

Hagemann TL, Assaád AH, Kwan S-P (1995): Mutation analysis of the gene encoding Bruton's tyrosine kinase in a family with a sporadic case of X-linked agammaglobulinemia reveals three female carriers. Am J Med Genet 59:188–192.

Hendriks RW, Mensink EJ, Kraakman ME, Thompson A, Schuurman RK (1989): Evidence for male X chromosomal mosaicism in X-linked agammaglobulinemia. Hum Genet 83:267–270.

Jin H, Webster AD, Vihinen M, Sideras P, Vorechovsky I, Hammarstrom L, Bernatowska Matuszkiewicz E, Smith CI, Bobrow M, Vetrie D (1995): Identification of Btk mutations in 20 unrelated patients with X-linked agammaglobulinaemia (XLA). Hum Mol Genet 4:693–700.

Kwan S-P, Kunkel L, Bruns G, Wedgwood RJ, Latt S, Rosen FS (1986): Mapping of the X-linked agammaglobulinemia locus by use of restriction fragment-length polymorphism. J Clin Invest 77: 649–652.

Kwan S-P, Terwilliger J, Parmley R, Raghu G, Sandkuyl LA, Ott J, Ochs H, Wedgwood R, Rosen F (1990): Identification of a closely linked DNA marker, DXS178, to further refine the X-linked agammaglobulinemia locus. Genomics 6:238–242.

Lederman HM, Winkelstein JA (1985): X-linked agammaglobulinemia: An analysis of 96 patients. Medicine 64:145–156.

Leickley FE, Buckley R (1986): Variability in B cell maturation and differentiation in X-linked agammaglobulinemia. Clin Exp Immunol 65:90–99.

Malcolm S, de Saint Basile G, Arveiler B, Lau YL, Szabo P, Fischer A, Griscelli C, Debre M, Mandel JL, Callard RE, Robertson ME, Goodship JA, Pembrey ME, Levinsky RJ (1987): Close linkage of random DNA fragments from Xq21.3-22 to X-linked agammaglobulinaemia (XLA). Hum Genet 77:172–174.

Mensink EJ, Thompson A, Schott JD, van der Greef WM, Sandkuyl LA, Schuurman RK (1986): Mapping of a gene for X-linked agammaglobulinemia and evidence for genetic heterogeneity. Hum Genet 73:327–332.

Rohrer J, Parolino O, Belmont JW, Conley ME (1994): The genomic structure of human BTK, the defective gene in X-linked agammaglobulinemia. Immunogenetics 40:319–324.

Tsukada S, Saffran DC, Raxlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S, Belmont JW, Cooper MD, Conley ME, Witte ON (1993): Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell 72:279–290.

Vetrie D, Vorechovsky I, Sideras P, Holland J, Davis A, Flinter F, Hammarström L, Kinnon C, Levinsky R, Bobrow M, Smith CI, Bentley DR (1993): The gene involved in X-linked agammaglobuliaemia is a member of the *src* family of protein-tyrosine kinases. Nature 361:226–233.

Vihinen M, Nilsson L, Smith E (1994): Structural basis of SH2 domain mutations in X-linked agammaglobulinemia. Biochem Biophys Res Commun 205:1270–1277.

Vihinen M, Cooper MD, de Saint Basile G, Fischer A, Good RA, Hendriks RW, Kinnon C, Kwan S-P, Litman GW, Notarangelo LD, Ochs HD, Rosen FS, Vetrie D, Webster AD, Zegers BJ, Smith CI (1995): BTKbase: A database of XLA-causing mutations. Immunol Today 16:460–465.

Vorechovsky I, Vihinen M, de Saint Basile G, Honsova S, Hammarstrom L, Muller S, Nilsson L, Fischer A, Smith CI (1995): DNA-based mutation analysis of Bruton's tyrosine kinase gene in patients with X-linked agammaglobulinaemia. Hum Mol Genet 4:51–58.

Wedgwood RJ, Ochs HD (1980): Variability in the expression of X-linked agammaglobulinemia: The co-existence of classic XLA (Bruton type) and "common variable immunodeficiency" in the same families. In: Seligman M, Hitzig WH (eds): "Primary Immunodeficiencies." INSERM Symposium No 16, Amsterdam, Elsevier, pp 69–78.